Influence of handling and conditioning protocol on learning and memory of *Microplitis croceipes*

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Abstract

Microplitis croceipes (Cresson) (Hymenoptera: Braconidae) learns odors in association with both hosts and food. The food-associated 'seeking' behavior of *M. croceipes* was investigated under various training protocols utilizing the conditioning odor, 3-octanone. We investigated the effects of odor training, or its lack, training duration, training frequency, time elapsed after training, wasp hunger state, and training reinforcement, on the food-seeking responses of *M. croceipes* females. We found that odor-trained females show strong food seeking responses, whereas non-odor-trained females do not respond to the odor, and that a single 10 s association with the odor whilst feeding on sugar water subsequently conditioned the wasps to exhibiting significant responses to it. Increases in training time to more than 10 s did not improve their responses. Repetition of the food–odor associations increased a wasp's recall, as well as its response over time, compared to a single exposure. Repeated exposure to the learned odor in the absence of a food reward decreased the responses of less hungry individuals. However, the level of response increased significantly following a single reinforcement with the food–odor association. Understanding the factors that influence learning in parasitoids can enhance our ability to predict their foraging behavior, and opens up avenues for the development of effective biological detectors.

Introduction

Parasitic wasps detect and learn numerous chemical cues associated with their host and food resources, and use them in order to forage more effectively (Lewis & Martin, 1990; Lewis et al., 1991; Zanen & Cardé, 1991). Recent studies of the potential of wasps as biological detectors has also indicated that *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a specialist parasitoid of three highly polyphagous hosts species, can also detect and learn 'foreign' chemicals that are unlikely to have played a role as foraging cues during their evolution (Wäckers et al., 2002; Olson et al., 2003). This learning ability elicits specific behavioral responses to cues associated with both long- (e.g., flight) and short-range (e.g., area restricted search) foraging behaviors (Olson et al., 2003). This species can also be trained to simultaneously associate two different odors with food and host rewards (Lewis & Takasu, 1990), or successively to two different odors with the same reward (Takasu & Lewis, 1996), suggesting that they are able to store this information for future use according to their specific resource needs. Although the ability of these species to detect and learn a wide variety of chemicals has been determined, we still have very little information on the factors that affect their acquisition and memory of learned experiences.

The optimal training protocol and age of females used for flight responses of this species after food associated conditioning were determined by Takasu & Lewis (1996); responses peak 2–5 days after emergence and after five, 30 s exposures to food and odor experience, which they remember for at least 2 days. Takasu & Lewis (1996) also showed that female *M. croceipes* will cease to respond to learned odors after several experiences with the odor without a food reward, and that they will switch to a different odor they had recently experienced as rewarding. However, the effect of a state of hunger on responses over time, and the factors which affect learning and memory of this species to cues associated with the more short-range behavioral responses are not known. We chose a food-associated seeking behavior of *M. croceipes* females, which is a modification of 'area restricted search' and other food-specific behaviors

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(Curio, 1976; Wäckers et al., 2002). Instead of intense local search and substrate antennation, we trained wasps to enter a hole from where the conditioned odor emanated (Olson et al., 2003).

Herein, we investigated the effects of odor conditioning or not, duration of exposure to odor and food, frequency of odor and food exposure, wasp hunger state, and training reinforcement on the food-associated seeking responses of *M. croceipes*.

Materials and methods

Microplitis croceipes was reared on *Heliothis zea* Boddie (Lepidoptera: Noctuidae) larvae according to the method described by Lewis & Burton (1970). Adult parasitoids were provided with water and honey, and kept in a Plexiglas cage ($30 \times 30 \times 17$ cm). Its environmental conditions were 28 °C, 50–70% r.h., and a L16:D8 photocycle. Female *M. croceipes*, 2–3 days old, mated without oviposition experience, were used in the experiments.

General food-associated training procedure

Female *M. croceipes* were conditioned with 3-octanone at a release rate of 2 $\mu g\ h^{-1}$ using a membrane loaded with pure chemical as described in Heath et al. (1996). The membrane with the 3-octanone was placed in a 45/50 RodaViss volatile collection chamber 25 cm long (Analytical Research Services Inc., Gainsville, FL; Figure 1C) with openings at each end. One opening was attached to a flow meter and a pump, and ambient air was pumped through the volatile collection chamber at a rate of 40 ml min^{-1}. The other end of the volatile collection chamber was attached to the arm of a 125 ml flask (Figure 1A). The opening of the flask was covered with aluminum foil, and a piece of filter paper (4 mm^2) saturated with the sucrose–water solution was placed on top and in the center of the aluminum foil (Figure 1A). A total of 7–9 holes (1 mm diameter) were punctured in the aluminum foil around the edge of the filter paper with the tip of a forceps so that the odor would flow through the holes, upward and around the filter paper as the wasp fed on the sugar water (Figure 1A). Each wasp was allowed to feed for a specified amount of time, as described below.

General testing procedure

The flask used in training was replaced by an acrylic cylinder (2 mm diameter) with a Teflon cover containing a 2 mm diameter hole through its center (Figure 1B). The 3-octanone (2 $\mu g\ h^{-1}$ release rate) was delivered to individual wasps through the Teflon cover in the cylinder at a flow

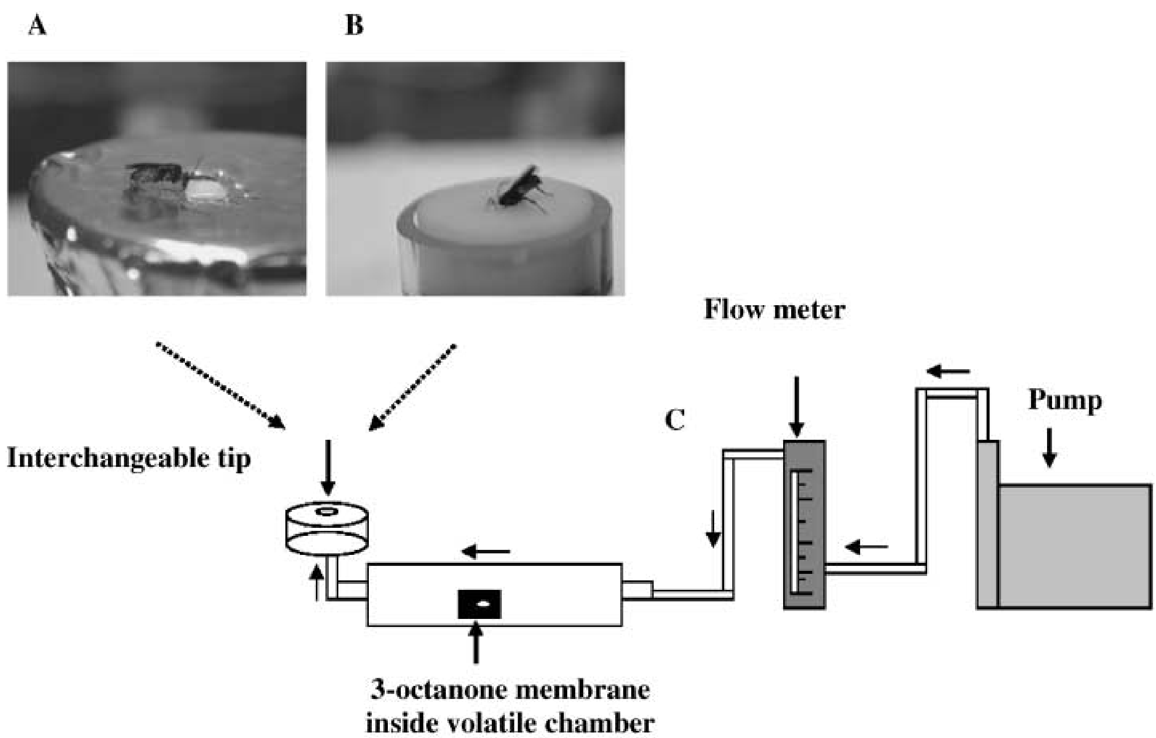

Figure 1 Diagram of the chemical delivery device (C) used to condition (A) (wasp feeds on sugar water with or without conditioning odor), and test food seeking responses (B) (conditioned wasp enters the hole where odor emanates).

rate of 5–10 ml min^{-1}. The wasps were released near the recessed hole of the Teflon cover, so their antennae could sense the odor. Females responded positively by entering the hole (at least half of their body length within the hole) (Figure 1B), immediately, or after intense substrate antennation. Females responded negatively by walking over the hole and around the Teflon cover and glass, remaining in the vial or flying away. Females that stopped the intense substrate antennation for 10 s were recorded as having no response. Based on preliminary observations, females that stopped substrate antennation for 10 s would not enter the hole after 1 min and often flew away. The flow rate was reduced to about 10 ml min^{-1} for testing, because preliminary tests indicated better responses at lower flow rates. All training and testing took place under a chemical fume hood with constant ventilation.

Effects of odor-conditioning or not on behavioral responses

To determine the robustness and fidelity of the responses of the wasps to the chemical odor, we tested the influence of odor conditioning, and no odor, with sucrose and water on the food-seeking responses of a large number of *M. croceipes* females from different cohorts.

Training. The female *M. croceipes* were 2–3 days old and had been provided water for 46–50 h at the time of experiment. Each female was allowed to feed on sucrose three times, for 10 s and 30 s intervals while 3-octanone was delivered at a 2 μg h^{-1} release rate.

Testing. A total of 10 odor conditioned and 10 sucrose-only conditioned females were tested each day for 4 days with a different cohort used each day. The influence of the day of conditioning and/or cohort (1–4), and conditioning treatment (trained and untrained) on the food-seeking responses of *M. crociepes* were tested with a 2×4 and a 2×2 contingency table analysis, respectively (SAS version 7.0, SAS Institute, 1998).

Effects of duration of odor-conditioning on behavioral responses

Training. To determine how the duration of food-odor exposure affected the food seeking responses of the wasps, five groups of female *M. croceipes* were trained a single time for 5 s, 10 s, 20 s, 30 s, and 40 s, respectively. A total of five females was trained and tested per group per day over 5 days, with a different cohort utilized each day, with each day representing a replicate.

Testing. The seeking response of individual wasps was tested to the conditioning odor 15 min after training. The influence of day conditioning (1–5) and duration of exposure (5 s, 10 s, 20 s, 30 s, and 40 s) on the food seeking responses of females were tested with a 2×5 contingency table analysis (SAS Institute, 1998).

Effects of frequency of odor conditioning on behavioral responses through time

Training. To determine if the frequency of exposures to food and odor affected the behavioral responses of the wasps over time, one group of female *M. croceipes* was trained for 10 s, and a second group was trained for 10 s, three times with a 30 s interval between exposures. A total of 10 females were trained per group per day over 4 days, with days representing replicates.

Testing. Both groups were tested for their response to 3-octanone, 15 min, 24 h, and 48 h after training. Females were returned to rearing cages with a water supply prior the 24 h and 48 h tests. Prior to the 48 h test, females were allowed to feed on sucrose water (50%) for 2 min, 24 h after training, to keep them alive, because they cannot survive more than 72 h without food (Takasu & Lewis, 1996). A total of 10 females was tested per group per day over 4 days, with a different cohort used each day. The influence of the day of experiment (replication), frequency of exposure (1 or 3 times), length of time after training (15 min, 24 h, or 48 h), and their interactions on the food seeking behavior of female wasps were tested using a linear model for contingency table analysis (SAS Institute, 1998). The dependence of the frequency of exposures on food seeking behavior over time was tested with a linear regression model, and a t-test was used to determine the difference between the slopes of the lines (SAS Institute, 1998).

Effects of hunger state and lack of reward on behavioral responses through time

To determine if hunger state influenced the behavioral responses of odor-conditioned females that had been repeatedly exposed to the odor without a food reward, we presented females with different feeding regimes prior to training and tested their seeking responses after initial training and each hour thereafter for 3 h.

Training. We controlled for differences in the emergence rates of female *M. croceipes* from a single cohort, and thus, their feeding history prior to training and testing, by utilizing randomly chosen females from a single cohort. Three groups of 10 females from the cohort were chosen and placed into three separate cages, and different feeding regimes were provided for each cage following wasp emergence. Wasps in the first cage were provided with water only for 48 h (= 2-day-old hungry wasps). Females in the second cage were provided water and honey for 24 h and then water only for 24 h (= 2-day-old well-fed wasps). Females in the last cage were provided water and honey for 24 h and water only for 48 h (= 3-day-old fed wasps). Females from each of the three feeding regimes were odor conditioned for the tests by allowing them to feed on sucrose and water in the presence of 3-octanone odor for three times 10 s, with 30 s intervals between feeding. A total of eight females was trained and tested per day over 4 days with days representing replicates.

Testing. Each female *M. croceipes* was tested four successive times, the first time between 5 and 10 min after training, the 2nd time 1 h after the initial test, and the 3rd and 4th times, 1 h after the 2nd and 3rd tests, respectively. The influence of the day of experiment (replication), hunger state (hungry, well-fed, and fed), the period of exposure after training (5–10 min, 1 h, 2 h, and 3 h), and their interactions, on the food seeking behavior of female wasps were tested using a linear model for contingency table analysis (SAS Institute, 1998).

Effects of reinforcement on behavioral responses

To determine if the decrease in the seeking response over time of the well-fed females was due to a lack of hunger, we used the same training and testing protocol as in the previous experiment, except that we provided a single reinforcement of sucrose feeding, with and without the 3-octanone after the third unrewarding experience, to test the influence of this reinforcement on well-fed wasp responses in the final (4th) test.

Training. Female *M. croceipes*, from a single 2-day-old cohort, that had fed on honey for 1 day and provided with only water on the second day were randomly chosen for initial training. For initial training, the wasps were fed on sucrose and water in the presence of 3-octanone vapor for three times 10 s, with 30 s intervals between feeding. After training and the initial test, the females were divided into two groups and placed into two separate cages and provided with only water for 1 h until the next test. Reinforcement was provided once, immediately prior to the 4th test. For the reinforcement, females from one cage were given a single 10 s sugar water feeding with 3-octanone odor, and the females from the other cage were given a single 10 s sugar water feeding without odor. A total of eight females was trained and tested per day over a total of 4 days.

Testing. Female *M. croceipes* were initially tested between 5 and 10 min after training, and each hour thereafter for 3 h. The influence of the day of experiment (replication), type of reinforcement (sucrose only and sucrose + odor), time of exposure after training (5–10 min, 1 h, 2 h, and 3 h), and their interactions on the food seeking behavior of female wasps were tested using a linear model for contingency table analysis (SAS Institute, 1998).

Results

Effects of odor conditioning or not on behavioral responses

There was no significant effect from the day of the experiment and/or the particular cohort on female *M. croceipes* responses ($\chi^2 = 0.1524$, d.f. = 3, $P = 0.985$; $n = 80$). There was a significant difference in female responses depending on conditioning ($\chi^2 = 62.22$, d.f. = 1, $P < 0.001$). Significantly more females elicited food seeking responses when conditioned with sucrose and odor, whereas none of the females given only sucrose elicited these responses (Figure 2).

Effects of duration of odor conditioning on behavioral responses

There was no significant effect from the day of the experiment on female *M. croceipes* responses ($\chi^2 = 1.86$, d.f. = 4, $P = 0.760$; $n = 125$). There was also no significant overall effect of the duration of exposure on the food seeking response of females (Figure 3; $\chi^2 = 7.837$, d.f. = 4, $P = 0.097$; $n = 125$), but a significantly lower number of wasps that had been exposed to the odor for only 5 s responded to it compared to those females exposed for 10, 20, 30, and 40 s (Figure 3.; $\chi^2 = 6.82$, d.f. = 1, $P < 0.009$; $n = 125$). A 10 s training session was sufficient to obtain responses close to 80%, and exposures longer than this did not increase the food seeking responses of females to 3-octanone (Figure 3).

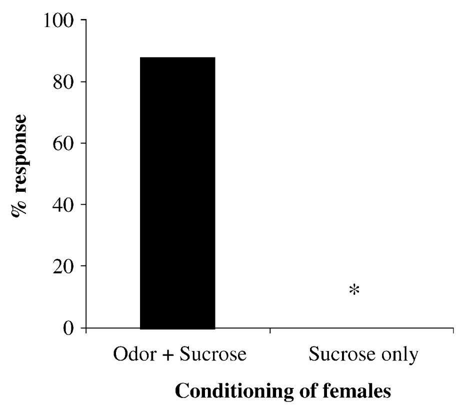

Figure 2 Hungry female *Microplitis croceipes* food seeking responses to 3-octanone (2 µg h^{-1} release rate) after odor conditioning with 3-octanone and sucrose and water, and conditioning with sucrose and water only. Asterisk above the bars indicates significant differences in responses between conditioning treatments at $P < 0.001$. Forty females were assayed per treatment.

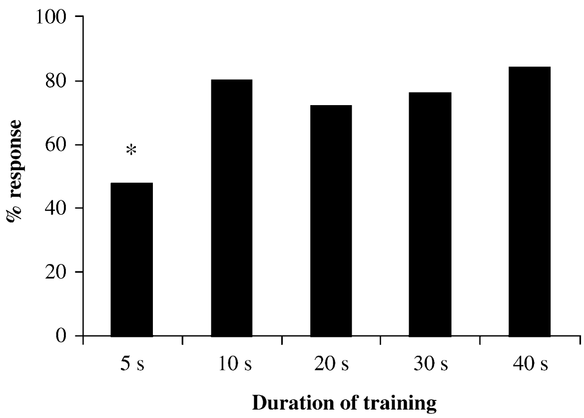

Figure 3 Influence of the duration of a single odor conditioning event on the seeking responses of female *Microplitis croceipes.* The female wasps were odor conditioned by exposing them to 3-octanone (2 $\mu g\,h^{-1}$ release rate) and food (sucrose water) for 5, 10, 20, 30, or 40 s. They were tested 15 min after odor conditioning, with a total of five females from each odor-conditioning treatment tested per day over 5 days. The asterisk above the bar indicates significant differences in responses between conditioning treatments at $P < 0.009$.

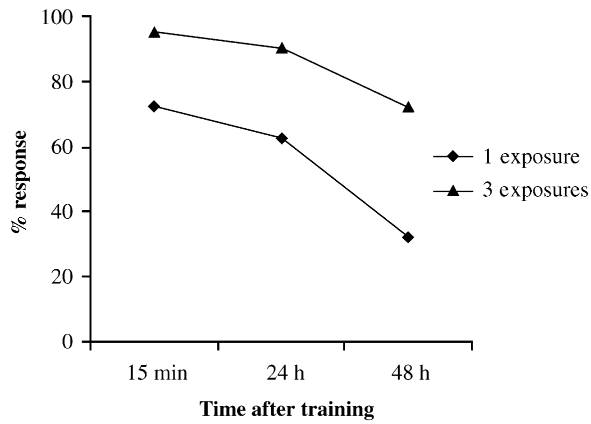

Figure 4 Influence of the frequency of odor conditioning (one or three exposures) and length of time after odor conditioning (15 min, 24 h, or 48 h), on the food seeking responses of female *M. croceipes.* Female wasps were odor conditioned by exposing them to 3-octanone at a 2 $\mu g\,h^{-1}$ release rate while feeding for 10 s (one exposure) or for three times 10 s (three exposures) with 30 s intervals between feeding. A total of 10 females in each conditioning treatment were tested per day over 4 days. Equations for the lines: one exposure $= -0.226x + 0.575$; $t = 5.72$, d.f. = 1, $P < 0.03$, and three exposures $= -0.113x + 0.858$; $t = 2.86$, d.f. = 1, $P > 0.10$).

Effects of frequency of odor-conditioning on behavioral responses through time

There was no significant effect from the day of the experiment (replication) on female *M. croceipes* responses (Table 1). The frequency of exposure to food and odor during training significantly influenced the seeking responses of the wasps (Table 1). Exposures to food and odor repeated three times, significantly improved the responses of females compared to their responses following a single exposure (Table 1, Figure 4). The rate of decrease varied between the frequency of exposures to the conditioning odor (Figure 4; one exposure $= -0.226x + 0.575$, $t = 5.72$, d.f. = 1, $P < 0.03$, and three exposures $= -0.113x + 0.858$, $t = 2.86$, d.f. = 1, $P > 0.10$).

Table 1 Contingency table analysis using a linear model to test the effects of day of experiment (= rep), number of exposures to odor and food (= treat: one and three exposures), time after training (= time: 15 min, 1 day, and 2 days), and their interactions on the food seeking behavior of *M. croceipes* females

Source	d.f.	χ^2	P-value
Treat	1	20.01	0.0001
Time	2	21.05	0.0001
Treat × time	2	0.00	0.9996
Rep	3	1.49	0.6838
Rep × treat	3	1.83	0.6083
Likelihood ratio	12	7.82	0.7988

Effects of hunger state and lack of reward on behavioral responses over time

There was an effect from the day of the experiment on the food seeking responses of female *M. croceipes* (Table 2). Significantly fewer ($\chi^2 = 41.39$, d.f. = 3, $P < 0.001$) females elicited food seeking behaviors on day 1 (65%, n = 91 responses measured; $\chi^2 = 32.21$, d.f. = 3, $P < 0.001$) compared to day 4 (74%, n = 92), whereas significantly more wasps elicited food seeking behaviors on day 2 (91%, n = 96; $\chi^2 = 10.07$, d.f. = 3, $P < 0.001$), and day 3 (84%, n = 96; $\chi^2 = 4.11$, d.f. = 3, $P < 0.043$), compared to day 4. There was a marginal significance for the overall hunger state and time after training interaction on food seeking responses (Table 2). However, an analysis of the likelihood estimates indicated significantly higher food seeking responses of well fed females in the initial test (5–10 min after training), compared to their responses in the last test (3 h after training) (Figure 5; $\chi^2 = 5.59$, d.f. = 6, $P < 0.018$).

Effects of reinforcement on behavioral response

There was a significant effect from the day of the experiment on the food seeking responses of female *M. croceipes* (Table 3). Females elicited fewer food seeking behaviors on day 2 (44%, n = 61 responses measured; $\chi^2 = 14.73$, d.f. = 3, $P < 0.001$), compared to those responses on day 4

Table 2 Contingency table analysis using a linear model to test the effects of day of experiment (= rep), hunger state (= treat: hungry, well-fed, and fed), time after training (= time: 5–10 min, 1 h, 2 h, and 3 h), and their interactions on the food seeking behavior of *M. croceipes* females

Source	d.f.	χ^2	P-value
Treat	2	32.44	0.000
Time	3	37.05	0.000
Treat × time	6	12.39	0.054
Rep	3	41.39	0.000
Rep × treat	6	12.55	0.051
Likelihood ratio	60	44.35	0.935

Table 3 Contingency table analysis using a linear model to test the effects of day of experiment (= rep), type of reinforcement (= treat: food + odor and food only), time after training (= time: 5–10 min, 1 h, 2 h, and 3 h), and their interactions on the food seeking behavior of *M. croceipes* females

Source	d.f.	χ^2	P-value
Treat	1	14.51	0.000
Time	3	72.62	0.000
Treat × time	3	9.25	0.026
Rep	3	16.36	0.001
Rep × treat	3	1.23	0.746
Likelihood ratio	39	33.88	0.702

(64%, n = 64). There was a significant interaction between time after training and reinforcement treatment on behavioral responses (Table 3). Although individual likelihood estimates did not indicate significant differences in females responses with respect to this two-way interaction, more odor-reinforced females (88%) showed food seeking responses in the 3 h test than those females that were not odor reinforced (41%; Figure 6), Overall, significantly more odor-reinforced females showed food-seeking behavior, compared to those females reinforced with only sucrose and water ($\chi^2 = 14.51$, d.f. = 1, $P < 0.001$). Significantly more food-seeking responses occurred in the first test ($\chi^2 = 41.46$, d.f. = 3, $P < 0.001$), and significantly fewer food seeking responses occurred in the 2 h test ($\chi^2 = 57.53$, d.f. = 3, $P < 0.001$) when compared to their responses in the 3 h test (Figure 6).

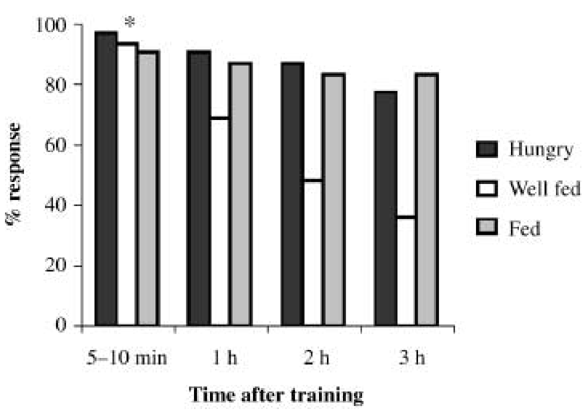

Figure 5 Influence of hunger state (hungry, fed, and well fed), and time of exposure to the conditioning odor (3-octanone at 2 μg h^{-1} release rate) after initial odor conditioning (5–10 min, 1 h, 2 h, and 3 h) on the seeking responses of female *M. croceipes.* Prior to the odor conditioning, female wasps were only provided with water for 48 h (hungry), honey and water for 24 h and water only for 24 h (well-fed), or honey and water for 24 h and water only for 48 h (fed). Females were odor conditioned by exposing them to 3-octanone while feeding on sucrose and water for three times 10 s with 30 s intervals between feeding. A total of eight females was odor conditioned and tested per day over 4 days. The asterisk above the white bar indicates significant differences in responses compared to the white bar in the 3 h test at $P < 0.001$.

Discussion

Our results show that no females conditioned with only sucrose and water responded to the chemical odor, whereas 88% of the odor-conditioned females elicited food-seeking responses to the odor. These results suggest that the behavioral assay was a very robust method for training and testing females that had been odor conditioned to 3-octanone.

Our results also show that *M. croceipes* females are able to effectively associate chemical odors to food with a single, brief exposure (10 s) to the odor whilst feeding. Wasps so trained, subsequently display food-seeking responses to the learned odor. Females exhibited a low response after a 5 s training session, and showed an optimum in response after a 10 s or longer training session. The frequency of the food and odor associations also affected their recall. *Microplitis croceipes* females that have a single, vs. three 10 s training sessions, showed comparable seeking responses 1 day after training. However, by 2 days after the training, response levels by individuals with only one exposure declined significantly more than those of females with three exposures of the food and odor association. These results suggested that *M. croceipes* wasps are able to learn odors in association with food within a very short training period (10 s), and that repetition improves their response level and memory.

Although there is a potential confounding effect of time of exposure and hunger state, we were able to demonstrate

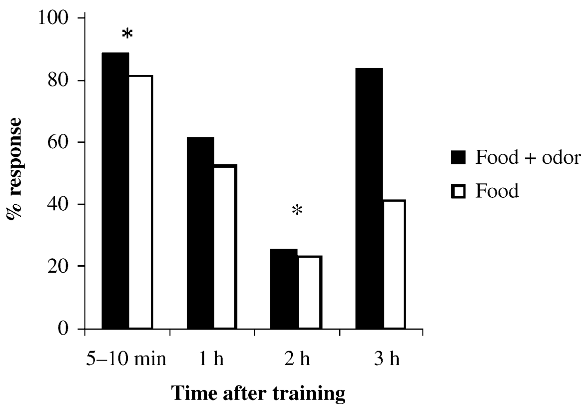

Figure 6 Influence of the time of exposure after initial odor conditioning (5–10 min, 1 h, 2 h, and 3 h) to 3-octanone (2 μg h^{-1} release rate), and reinforcement type (food + odor and food only), on the seeking responses of female *M. croceipes.* Females from a single cohort were provided with honey for 24 h and water only for the next 24 h prior to initial conditioning (= well-fed). Initial conditioning consisted of three times 10 s with 30 s interval exposures to 3-octanone (2 μg h^{-1} release rate) while feeding on sucrose and water. For reinforcement, females from one cage were given one 10 s sucrose and water feeding with 3-octanone odor, and females from the other cage were given a single 10 s sucrose and water feeding without odor. A total of eight female wasps was odor conditioned and tested per day over 4 days. Asterisks above the black and white bars indicate significant differences in responses when reinforcement type is compared to the responses in the 3 h test at $P < 0.001$.

that hunger state modulates the effect of repeated exposure to the conditioning odor in the absence of any food reward. Females that were relatively hungry (hungry and fed wasps) maintained high seeking responses when repeatedly exposed to the conditioning odor without a food reward. In contrast, the responses of less hungry females rapidly decreased with unrewarded exposures to the conditioning odor. More interestingly, our results show that the decreased responses of the less hungry females could be restored with a single odor training reinforcement. This suggested that the females eventually forget or habituate to the compound when they are not rewarded and not satiated, but elevated seeking responses are recovered with a single brief exposure to the odor and food. It would be interesting to determine if the responses of well-fed females changed over similar time intervals without the potential confounding effect of having had previous negative experiences.

The day of the experiment had significant effects on the wasps' responses in two of the experiments, suggesting that there are other factors that we did not control for that can influence the behavior of this wasp species. Variances in behavior from day-to-day can have many sources, including ambient environmental conditions, individual genetics, and finer scale differences in hunger state resulting from the non-uniform emergence. We would need to better control for these and other factors to determine their influence on the food seeking behavior of *M. croceipes*.

Previous studies found that flight choices for an odor learned in association with food by *M. croceipes* females (Takasu & Lewis, 1996), and the proboscis extension by honeybees (Getz & Smith, 1991; Smith, 1998) increases when they experienced repeated exposures to food and odor during training. *Microplitis croceipes* decrease their responses after 10 repetitions of exposure to the conditioning odor without reward, and honeybees show an extinction of responses without reward. However, the *M. croceipes* wasps so handled, continued the general search for food and fed further afield when food was located (Takasu & Lewis, 1996), suggesting that, similar to our study, habituation to or forgetting the conditioned odor, rather than satiation, was the reason for the decreased flight choice responses. Moreover, other studies have indicated that the hunger state of odor-conditioned *M. croceipes* affects their flight behavioral responses (Lewis & Takasu, 1990; Takasu & Lewis, 1993); female wasps are more sensitive to food-associated odors in flight assays if they have increased hunger levels. Trained *M. croceipes* females also fly upwind to host- and food-associated odors based on their physiological state; hungry females prefer odors associatively linked to food over host-associated odors. In contrast, well-fed females trained to odors with hosts prefer host-linked odors over food-associated odors (Takasu & Lewis, 1993). Similarly, the hungry females in our study continued to respond to food-associated odors regardless of the number of unrewarded experiences, whereas less hungry females ceased responding after a few unrewarding experiences. Although not tested here, it would be interesting to determine if the more hungry females would respond to any odor because they were hungry or if they only show high fidelity to the conditioning odor. The results from this study are also in agreement with studies in honeybees showing that the time elapsed since training (Smith, 1991) and reinforcement after unrewarding experiences (Getz & Smith, 1991) are important in eliciting and maintaining behavioral responses to the trained odor. Thus, responses can vary with the post-conditioning time, and number of unrewarding exposures. More studies are needed to determine the optimal period between training and testing, and optimal time and amount of reinforcement, to be able to predict behaviors of specific species after training. Understanding the factors that influence learning in parasitoids enhances our ability to predict their foraging behavior, as well as our ability to develop effective biological detectors.

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